

ESSAY REVIEW

Atmosphere, ecology and evolution: what drove the Miocene expansion of C₄ grasslands?

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Summary

1. Grasses using the C₄ photosynthetic pathway dominate today's savanna ecosystems and account for ~20% of terrestrial carbon fixation. However, this dominant status was reached only recently, during a period of C₄ grassland expansion in the Late Miocene and Early Pliocene (4–8 Myr ago). Declining atmospheric CO₂ has long been considered the key driver of this event, but new geological evidence casts doubt on the idea, forcing a reconsideration of the environmental cues for C₄ plant success.
2. Here, I evaluate the current hypotheses and debate in this field, beginning with a discussion of the role of CO₂ in the evolutionary origins, rather than expansion, of C₄ grasses. Atmospheric CO₂ starvation is a plausible selection agent for the C₄ pathway, but a time gap of around 10 Myr remains between major decreases in CO₂ during the Oligocene, and the earliest current evidence of C₄ plants.
3. An emerging ecological perspective explains the Miocene expansion of C₄ grasslands via changes in climatic seasonality and the occurrence of fire. However, the climatic drivers of this event are debated and may vary among geographical regions.
4. Uncertainty in these areas could be reduced significantly by new directions in ecological research, especially the discovery that grass species richness along rainfall gradients shows contrasting patterns in different C₄ clades. By re-evaluating a published data set, I show that increasing seasonality of rainfall is linked to changes in the relative abundance of the major C₄ grass clades Paniceae and Andropogoneae. I propose that the explicit inclusion of these ecological patterns would significantly strengthen climate change hypotheses of Miocene C₄ grassland expansion. Critically, they allow a new series of testable predictions to be made about the fossil record.
5. *Synthesis.* This paper offers a novel framework for integrating modern ecological patterns into theories about the geological history of C₄ plants.

Key-words: atmospheric CO₂, C₄ plants, climate change, fire, grassland, grazing, Poaceae, rainfall, savanna, seasonality

Carbon dioxide and the expansion of C₄ grasslands

Major contrasts in the climatic preferences of grass subfamilies have been noted for more than half a century (Hartley 1950), but their significance was only recognized following the discovery of C₄ photosynthesis. Tropical and subtropical grasslands are dominated by the predominantly C₄ Panicoideae and Chloridoideae (Hartley 1958a,b; Hartley & Slater 1960), which together account for more than half of the world's grass

species (Fig. 1; Linder & Rudall 2005) and ~20% of gross terrestrial carbon fixation (Lloyd & Farquhar 1994). Their carbon-concentrating mechanism suppresses the energetically wasteful process of photorespiration that plagues C₃ grasses at high temperatures, and significantly raises the efficiency of photosynthesis in warm climate regions. In contrast, C₃ grass subfamilies such as the Pooideae are largely confined to temperate climates, where photorespiration is naturally limited by lower temperatures (Hartley 1961, 1973).

The evolutionary history of these patterns was elucidated only in the past two decades, following the realization that C₄ photosynthesis imparts a distinctive carbon isotope signature to plant materials and trophic pathways. By analysing the isotopic composition of fossilized soils (palaeosols) and the

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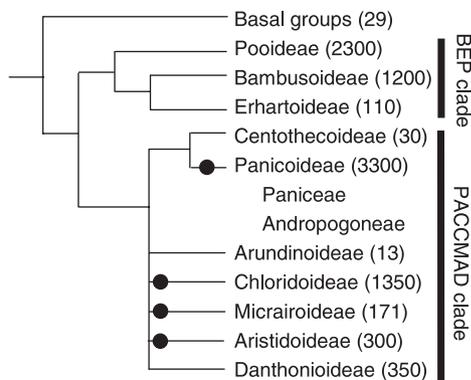


Fig. 1. Cladogram displaying the hypothesized relationships among subfamilies of the Poaceae based on multiple markers, with the number of species shown for each, and filled circles (●) indicating the unequivocal origins of C₄ photosynthesis in independent clades (Watson & Dallwitz 1992; GPWG 2001; Linder & Rudall 2005; Sánchez-Ken *et al.* 2007). Up to eight independent origination events may have occurred within the Panicoideae.

teeth of herbivores, geochemists uncovered a surprise; the domination of low-latitude ecosystems by C₄ grasses is a recent phenomenon in geological terms, occurring only 4–8 Myr ago (Ma) at the Miocene–Pliocene boundary, when C₄ grasslands expanded across at least four continents (Fig. 2; Quade *et al.* 1989; Cerling *et al.* 1997). The factors behind this evolutionary phenomenon have remained controversial since its discovery.

At first, debate focused on the relative merits of CO₂ as a driver of C₄ grassland expansion (e.g. Cerling *et al.* 1994; Morgan *et al.* 1994a,b). The case for CO₂ was persuasive, and based on the premise that selective and competitive advantages of C₄ photosynthesis result from the energetic benefits of eliminating photorespiration at high temperatures and low atmospheric CO₂ (Ehleringer *et al.* 1991, 1997; Cerling *et al.* 1997). These advantages over the ancestral C₃ condition are reversed when photorespiration is naturally suppressed by low temperatures or high CO₂, because the C₄ carbon-concentrating mechanism requires energy. This leads to critical thresholds of temperature (around 20–25 °C at today's atmospheric CO₂ concentration) and CO₂ (around 500 p.p.m. in tropical environments) where C₃ and C₄ photosynthesis have equal energy requirements and, by extension, equal competitive and selective advantages (Cerling *et al.* 1997). The proponents of this elegant hypothesis noted the close correspondence between the theoretical temperature threshold and mean growing season value in modern regions of equal C₃ and C₄ grass species richness, and postulated that declining CO₂ crossed an equivalent threshold at the Miocene–Pliocene boundary (Ehleringer *et al.* 1991, 1997; Cerling *et al.* 1997). Their ideas were supported by a geochemical model of atmospheric CO₂, which indicated the necessary decline during the Cenozoic (Berner 1998).

By the late 1990s, the ideas underpinning the CO₂ starvation hypothesis were widely accepted, but still awaited the crucial test provided by palaeo-CO₂ reconstructions. This soon came

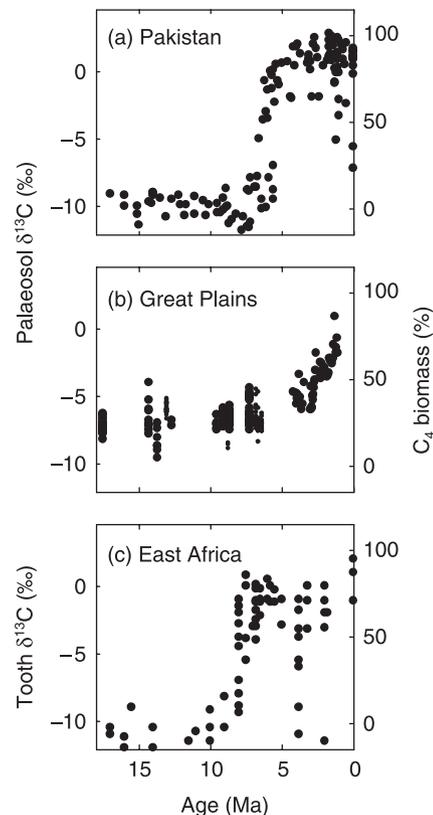


Fig. 2. Examples of the shifts in stable carbon isotope ratio (δ¹³C) characterizing the Miocene rise of C₄ plants in (a) Pakistan (Quade & Cerling 1995), (b) the Great Plains (Fox & Koch 2003) and (c) East Africa (Cerling *et al.* 1997). Values for (a) and (b) were obtained from palaeosol carbonates, and the proportion of biomass contributed by C₄ plants calculated following Fox & Koch (2003). Values for (c) are from the tooth enamel of mammalian herbivores, with the proportion of C₄ plants in their diets after Cerling *et al.* (1997).

with the publication of three data sets (Pagani *et al.* 1999; Pearson & Palmer 2000; Royer *et al.* 2001), each using an independent proxy for CO₂, and each showing a long period of stasis in the level of atmospheric CO₂ during the expansion of C₄ grasslands (Fig. 3b; reviewed by Royer 2006). Unless these palaeo-CO₂ proxy records are challenged on technical or theoretical grounds, the geological evidence therefore stands firmly against the CO₂ starvation hypothesis for C₄ grassland expansion, and new mechanisms must be sought. Instead, the latest evidence suggests that atmospheric CO₂ dropped sharply through the C₃–C₄ ‘crossover threshold’ at 25–30 Ma during the Oligocene, when it initiated our modern ‘icehouse’ era of advancing and retreating polar ice sheets (Fig. 3; Pagani *et al.* 2005; Royer 2006). The emerging picture of palaeoenvironmental change therefore lends credence to an alternative hypothesis, proposing declining CO₂ concentration as a key selection pressure for the evolutionary origins of C₄ photosynthesis in the grasses, rather than C₄ grassland expansion (Ehleringer *et al.* 1991; Pagani *et al.* 2005).

Here, I review two major issues currently facing geologists, physiological ecologists and ecosystem scientists with interests in this field. I first evaluate the proposed role of CO₂

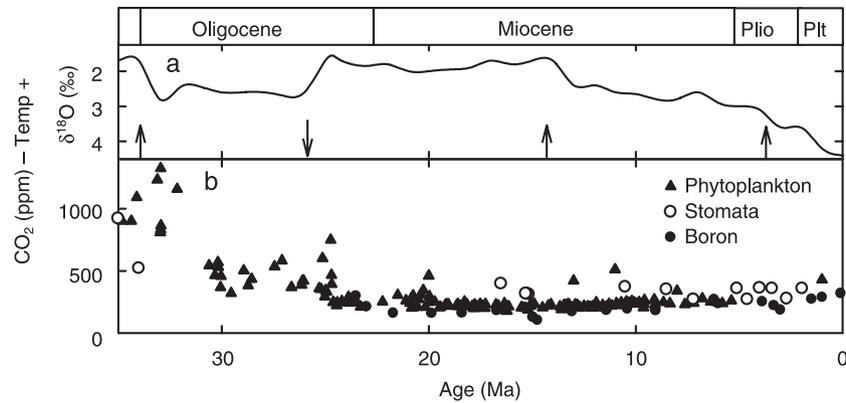


Fig. 3. CO₂ and climate change from the Oligocene to the present day. (a) Oxygen isotope values of deep-sea foraminifera ($\delta^{18}\text{O}$) displayed as a locally weighted running mean calculated by Zachos *et al.* (2001). This serves as a combined proxy for the global deep-sea temperature and continental ice volume, with increases in $\delta^{18}\text{O}$ indicating cooling and ice growth. Periods of rapid ice sheet growth are indicated by 'up' arrows, and contraction by 'down' arrows. The geological timescale shows ages in Myr ago (Ma); Plio, Pliocene; Plt, Pleistocene. (b) CO₂ proxy data based on carbon isotope ratios of marine phytoplankton, stomatal densities of fossil leaves and the boron isotope ratios of planktonic foraminifera compiled by Royer (2006). Palaeosol proxy data are not shown, because the uncertainty in this technique (± 500 p.p.m.) precludes meaningful estimation of low CO₂ levels.

in the evolutionary origins of *C*₄ photosynthesis, focusing on the question of when the pathway first arose in the grasses. However, my principal focus is on the current debate surrounding the causes of *C*₄ grassland expansion, particularly the hypothesized effects of climate change and fire. I argue that a deeper understanding of these proposed abiotic drivers could be achieved by explicitly considering their contrasting interactions within independent groups of *C*₄ grasses.

Dating the origins of *C*₄ photosynthesis

A crucial test of the CO₂ hypothesis for *C*₄ grass origins is to establish whether the first appearance of the pathway coincides with the Oligocene drop in atmospheric CO₂. Using a molecular clock, Gaut & Doebley (1997) calculated that divergence of two major *C*₄ grass tribes, the Paniceae and Andropogoneae (Fig. 1), occurred 25–32 Ma, leading later authors to argue that origins of *C*₄ photosynthesis must therefore be at least this old (e.g. Kellogg 1999). However, subsequent phylogenetic analysis cast doubt on this idea, suggesting that *C*₄ origins may have post-dated the divergence event, evolving in up to eight independent subgroups of the Paniceae and Andropogoneae (Giussani *et al.* 2001; Aliscioni *et al.* 2003; Sage 2004). Dating the nodes of the grass phylogenetic tree (Fig. 1) has proved difficult because it is becoming increasingly clear that variation in the mutation rate among branches generates large errors in molecular clocks (Pulquíero & Nichols 2007). Attempts at calibration using the fossil record have yielded dates that vary hugely because of the low sample sizes used (Linder & Rudall 2005). More precise dating of evolutionary events within the grass family therefore depends crucially on a better resolved fossil record.

Fossils only provide minimum dates for nodes of the phylogenetic tree because the geological record is incomplete. This problem is particularly acute for grasses of open habitats, where the potential for fossilization is extremely low (Cerling 1999). The oldest *C*₄ grass macrofossils date to only 12.5 Ma

in the Middle Miocene (reviewed by Osborne & Beerling 2006). However, the pollen record for grasses overall stretches back more than 65 Myr to the Late Cretaceous (Jacobs *et al.* 1999), indicating the potential for much earlier *C*₄ origins. Grass pollen cannot be identified below the family level, but phytolith traits map onto the grass phylogeny at the subfamilial scale (Piperno & Sues 2005; Prasad *et al.* 2005). Phytoliths form, to a varying degree, in all groups of living vascular plants (Piperno 2006). However, grasses have a higher silica production than most other plants (Hodson *et al.* 2005), and phytoliths in this family are more diagnostic than for most other plant groups. The interpretation of fossil phytolith assemblages is complicated by multiplicity (each species produces more than one phytolith morphotype), and redundancy (different species produce similar morphotypes) (Piperno 1988). Nevertheless, the comparison of fossil assemblages with modern reference collections has allowed robust statistical inferences about the phylogenetic affinities and ecology of extinct grass communities (Strömberg 2004, 2005).

Phytolith analysis has been used to identify grasses with affinities to the PACCMAD crown group (Fig. 1) from 65- to 67-Myr-old dinosaur dung in India (Prasad *et al.* 2005), and from the predominantly *C*₄ subfamily Chloridoideae (Fig. 1) in 19-Myr-old Great Plains sediments (Strömberg 2005). Although this technique cannot establish when *C*₄ photosynthesis originated in each clade, complementary methods have been developed for analysing the carbon isotopic signature of phytolith assemblages (Smith & White 2004). Interpretation of these data is difficult because of interspecific variation and differences in the biochemical nature of carbon compounds between *C*₃ and *C*₄ grass phytoliths (reviewed by Smith & White 2004). However, preliminary data suggest that up to 50% of Great Plains grasses may have used the *C*₄ pathway by 12 Ma (Smith 2001).

Geochemical analyses of herbivore teeth, palaeosols and the molecular markers of plant cuticles trace the *C*₄ carbon

isotope signal back to 16–18 Ma in the Early Miocene (reviewed in Tipple & Pagani 2007). These data are consistent with the presence of C₄ grasses comprising up to 30% of the total biomass in tropical and subtropical ecosystems throughout the Miocene (Fox & Koch 2003; Pagani *et al.* 2005; Tipple & Pagani 2007). However, the use of bulk isotope analyses to estimate C₄ biomass in extinct plant communities is not a precise science. Critical uncertainties remain about the isotopic composition of atmospheric CO₂ through geological time, variation in the background signal caused by the water relationships of C₃ plants and the taxonomic identity of the C₄ plants (Tipple & Pagani 2007).

Partial resolution of these issues may come from a new analytical technique for measuring the carbon isotope composition of tiny samples, which currently allows C₃ or C₄ pathways to be identified from individual pollen grains with > 85% reliability (Nelson *et al.* 2007). This emerging method should enable researchers to establish directly the photosynthetic pathway of parent plants using grass pollen recovered from sediments dating to the Early Miocene and Oligocene. Unlike analyses of palaeosol or tooth carbon, this technique does not require abundant C₄ plant biomass to resolve a C₄ signal from the C₃ background and offers the potential for identifying rare C₄ plants in a predominantly C₃ community, in addition to allowing identification of taxa to at least the family level. Furthermore, because it contrasts ‘pure C₃’ with ‘pure C₄’ samples, the technique is not compromised by variations in the isotopic signature of atmospheric CO₂. Pollen-based evidence may therefore bridge the gap between postulated C₄ origins and our oldest current evidence for C₄ plants. However, questions will still remain over the precise taxonomic identity of these plants, and pinning down the earliest C₄ grasses is only part of the challenge confronting

geologists in this field. Plummeting CO₂ concentrations were correlated with a whole suite of climatic changes during the Oligocene, including falling temperature and increasing aridity (Fig. 3; Zachos *et al.* 2001; Dupont-Nivet *et al.* 2007), and may not have been the only selection pressure for C₄ photosynthesis.

Hypothesized drivers of C₄ grassland expansion

Geological evidence therefore raises two linked questions. If CO₂ was not the driving force, what caused the Miocene–Pliocene expansion of C₄ grasslands? And, if C₄ grasses were present from the Early Miocene onwards, why did they not dominate ecosystems earlier in their evolutionary history? The key to answering both of these questions may lie in fossil evidence that documents an abundance of woody C₃ plants throughout the Miocene, forming forests, woodlands or savannas in regions that subsequently became C₄ grasslands (reviewed by Osborne & Beerling 2006). Woody plant cover exerts a major limitation on C₄ grass abundance, because trees and shrubs rapidly overtop herbaceous plants in the absence of disturbance, and most C₄ species are intolerant of shading (Sage *et al.* 1999; Sage & Pearcy 2000). Explanations of C₄ grassland expansion in the geological record therefore evoke a combination of climatic and disturbance factors that reduce tree cover, focusing primarily on changes in rainfall patterns and fire regime.

A number of alternative palaeoclimate hypotheses have been developed. The first is supported by the oxygen isotope signature of palaeosols in South Asia, and proposes that the Miocene replacement of C₃ woody vegetation by C₄ grasslands was driven by a strong increase in rainfall seasonality, caused by abrupt intensification of monsoon systems (Table 1;

Table 1. Comparison of the key hypotheses evoked to explain the Miocene–Pliocene expansion of C₄ grasslands. Alternative hypotheses are not mutually exclusive, and overlap to a significant extent. For example, a change in the fire climate could also be the ultimate reason for an ecotone shift (Keeley & Rundel 2003, 2005).

Hypothesis	Climatic seasonality	Climatic drying	Ecotone shift	Fire climate change
Ultimate reason for Miocene–Pliocene C ₄ grassland expansion	Development of seasonal climates with hot wet season and a dry season	Decrease in total rainfall without significant shifts in seasonality	C ₄ grasses evolved characteristics that allow displacement of woody plants from mesic habitats	Development of seasonal climates promoted fires
Mechanism of C ₄ grassland expansion	Woody plant mortality during annual dry season	Intensified drought events increased woody plant mortality	Shift in the C ₄ grass–woody plant ecotone to wetter (mesic) habitats	Increased frequency of fire prevented woody plant establishment
Why did C ₄ grasses not dominate ecosystems earlier in the Miocene?	Aseasonal climates favoured woody species	Wet climates favoured woody species	C ₄ grasses had not yet evolved characters allowing domination of mesic habitats	Infrequent fires allowed establishment of woody plant communities
Region	South Asia	South Asia, Africa	South Asia, Africa, North America	South Asia, China, West Africa
Key evidence	Palaeosol ¹⁸ O ratios Monsoon indicators	Freshwater bivalve ¹⁸ O ratios (rainfall seasonality) Model simulations	Palaeosol structure	Charcoal records Analogy with modern ecosystems
References	Quade <i>et al.</i> (1989, 1995)	Dettman <i>et al.</i> (2001), Sepulchre <i>et al.</i> (2006)	Retallack (2001)	Keeley & Rundel (2003, 2005)

reviewed by Osborne & Beerling 2006). A second hypothesis based on oxygen isotope data from freshwater bivalve shells in Nepal suggests a decrease in the total amount of rainfall with no significant change in seasonality (Dettman *et al.* 2001), and a similar mechanism is evoked for East Africa, rooted in model simulations for the region (Table 1; Sepulchre *et al.* 2006). Both of these climatic drying hypotheses propose that an increased frequency and intensity of drought events killed trees and allowed the incursion of *C*₄ grasses, to produce more open savanna or grassland vegetation (Table 1). Both are linked ultimately to tectonic events, including episodes of mountain building such as the uplift of the Tibetan Plateau and East African Rift system, or changes in ocean circulation triggered, for example, by closure of the seaway between the Americas (reviewed by Osborne & Beerling 2006).

An alternative viewpoint has emerged from analyses of palaeosol structure, which use the soil horizon structure to infer a vegetation type of desert, grassland or woodland, and the depth of the calcic horizon to estimate rainfall amounts (Retallack 2001). Application of this approach to geological sediments suggests that spatial gradients in rainfall generated parallel regional variation in vegetation (Fig. 4). Ecosystems varied from desert grassland to dry woodland throughout the Early and Middle Miocene in South Asia, the central and western United States and East Africa (Fig. 4; reviewed by Retallack 2001). Palaeobotanical evidence from East Africa also indicates savanna and woodland vegetation during the Early and Middle Miocene (Jacobs *et al.* 1999). However, the inference of dry climates is not supported by plant macrofossils, phytoliths and pollen from the Great Plains, which indicate productive savanna or woodland vegetation with a significant *C*₃ grass component (Jacobs *et al.* 1999; Strömberg 2004, 2005). The presence of palms, ginger and bamboos (Strömberg 2004), woody dicots confined to moister climates today (Axelrod 1985), and giant tortoises and alligators (Hutchinson 1982) suggests a relatively humid, rather than arid, climate. Palaeosol data suggest a major change in the ecology of these ecosystems during the Late Miocene, with *C*₄ grasses displacing woodland communities in mesic regions and shifting the grassland–woodland ecotone to higher rainfall areas in North America, Africa and Asia (Table 1, Fig. 4; Retallack 2001). The causes of this ecotone shift are unknown, but have been attributed to the coevolution of grasses and grazers (Retallack 2001), and significant changes in fire regime resulting from shifts in the seasonal distribution, but not total amount, of rainfall (Keeley & Rundel 2005).

Evidence supporting a linkage between fire and the expansion of *C*₄ grasslands comes from black carbon (BC) abundance in marine sediments, a geological proxy for fire occurrence. BC increases by 100- to 1000-fold in Pacific Ocean localities downwind of the Indian subcontinent (Herring 1985; Keeley & Rundel 2003), and 5-fold in the South China Sea (Jia *et al.* 2003), during the Miocene–Pliocene interval of major *C*₄ grassland expansion in these regions. Sediments off the Atlantic coast of West Africa also show significant increases in charred grass cuticle and pollen

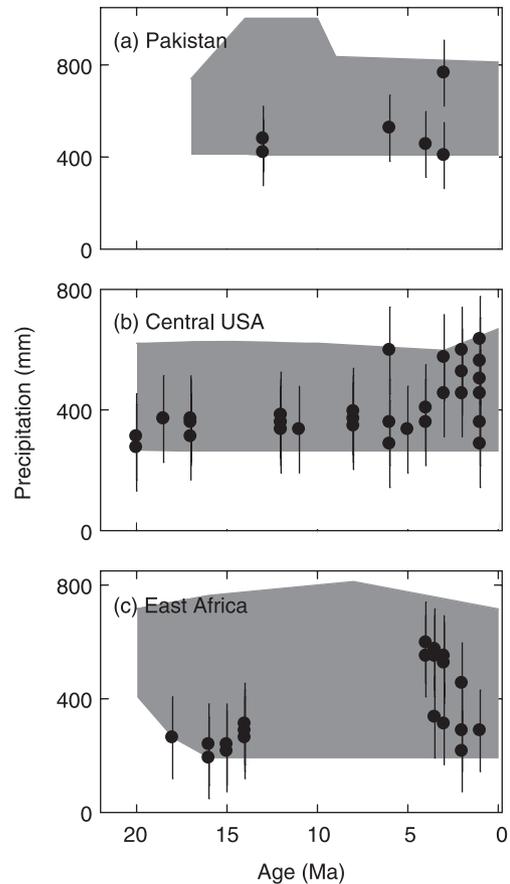


Fig. 4. Precipitation estimates based on depth to the calcic horizon in palaeosols from (a) Pakistan, (b) the central USA and (c) East Africa (Retallack 2001). The grey-shaded area denotes the total range of values obtained for all palaeosol types (originating in deserts, grasslands, woodlands and forests), and the symbols show values obtained for mollic palaeosols, which develop only beneath sod-forming grasses (Retallack 2001). These data suggest that grasslands invaded wetter areas from 8 Myr ago (Ma).

abundance during the same period (Morley & Richards 1993). In the last 5 years, a new and compelling hypothesis has therefore added ecosystem-scale feedbacks between fire and vegetation to previous ideas about palaeoclimate (Table 1; Keeley & Rundel 2003, 2005).

Fire sustains *C*₄ grasslands by killing woody plants and entrains a positive feedback because the dead foliage of grasses provides abundant fuel for fires, thereby increasing tree mortality and promoting the further spread of grasses (Keeley & Rundel 2003, 2005). The fire hypothesis therefore proposes that increasing climatic seasonality during the Late Miocene raised fire frequency by supporting rapid biomass production and the development of a high fuel load during wet summer conditions. Intensifying winter drought promoted drying, and increased the likelihood that this material would ignite, with ignition itself provided by lightning strikes at the end of the dry season. Further feedbacks on the fire regime have been proposed via the hydrological cycle (Beerling & Osborne 2006) and wind strength (Tippie & Paganini 2007), but these remain speculative at present.

Ecosystem fire regimes show an important interaction with climate. Fuel remains too moist to support frequent fires in wet, aseasonal environments, whilst the low productivity of dry climate regions produces too little fuel to carry significant fires (Keeley & Rundel 2005). However, given sufficient periods of dry weather, fire has the potential to displace mesic forests in favour of grasslands. This is demonstrated in modern ecosystems by complementary data from: model simulations showing that fire reduces forest cover by ~50% at the global scale (Bond *et al.* 2005); analysis of woody plant cover across Africa indicating that significant areas of savanna are maintained by disturbance in regions with sufficient rainfall to support forest (Sankaran *et al.* 2005); fire exclusion experiments which allow the establishment of fire-sensitive trees in mesic savanna ecosystems (Bond *et al.* 2003, 2005); and the fire-mediated replacement of native forests on Pacific Islands by invasive grasses (D'Antonio & Vitousek 1992). These observations each provide indirect support for the hypothesized mechanism of vegetation change at the Miocene–Pliocene boundary.

An emerging consensus therefore evokes interactions between palaeoclimate change, vegetation–climate relationships and fire frequency to explain the Miocene expansion of C_4 grasslands (Table 1; Keeley & Rundel 2005). This 'consensus scenario' suggests that Early and Middle Miocene landscapes were dominated by C_3 forests or woodlands, with C_4 grasses occupying open ground between the patches of woody plants. Increasing seasonality during the Late Miocene concentrated rainfall into a hot growing season, creating a fire regime that removed woody vegetation and shifted the woodland–grassland ecotone to wetter areas (Table 1). Formulated in this way, the hypothesis asserts that climates capable of supporting frequently burning mesic C_4 grasslands at the expense of woodland and forests were absent during the Early and Middle Miocene.

However, application of the hypothesis to North and South America is problematic in two important respects. First, patterns of climatic change in these regions at the Miocene–Pliocene boundary are less clear than those in Asia, and an increase in rainfall seasonality has yet to be demonstrated (Passey *et al.* 2002; Fox & Koch 2004; Osborne & Beerling

2006). Secondly, pollen records, phytolith assemblages and mammalian tooth morphology suggest that a transition from closed forest to open woody vegetation with a significant C_3 grass component may have occurred as early as 25 Ma (Late Oligocene to Early Miocene) in the Great Plains (Strömberg 2004, 2005) and 32 Ma (Early Oligocene) in South America (Jacobs *et al.* 1999). The adaptive radiation of specialist grazers (Strömberg 2006) and an extremely high browser diversity in these Great Plains ecosystems resulted in species-rich mammalian herbivore communities by 15 Ma (Janis *et al.* 2000). Carbon isotope data demonstrate that some grazers showed a dietary shift in the Late Miocene, subsisting almost exclusively on C_4 plants for the 2 Myr before C_4 grassland expansion, with presumably significant, but currently unknown, effects on grass–tree dynamics (Fox & Koch 2004; Beerling & Osborne 2006).

I argue that our overall understanding and ability to test climatic and ecological hypotheses regarding Miocene ecosystems would be improved by an explicit consideration of C_4 grass phylogeny. To make this case, I first present biogeographical data indicating significant contrasts in the abundance of different C_4 grass clades along rainfall gradients, and show how this information might be used in hypothesis testing. Secondly, I re-analyse a published data set in the same context, suggesting that changes in the species richness of independent C_4 grass lineages track the length of the rainy season. Finally, I present intriguing preliminary evidence of fire controls on the abundance of particular C_4 clades.

Contrasting climate relationships of C_4 grass clades

The subfamilies Panicoideae and Chloridoideae together account for the majority of modern C_4 grass species (Fig. 1; Linder & Rudall 2005), but analyses of regional biodiversity patterns suggest a crucial difference in the climate relationships of these major clades. For the United States and Argentina, the percentage of C_4 species belonging to the Panicoideae shows a positive correlation with annual rainfall, whereas the same relationship for the Chloridoideae is negative (Fig. 5; Taub 2000; Cabido *et al.* 2007).

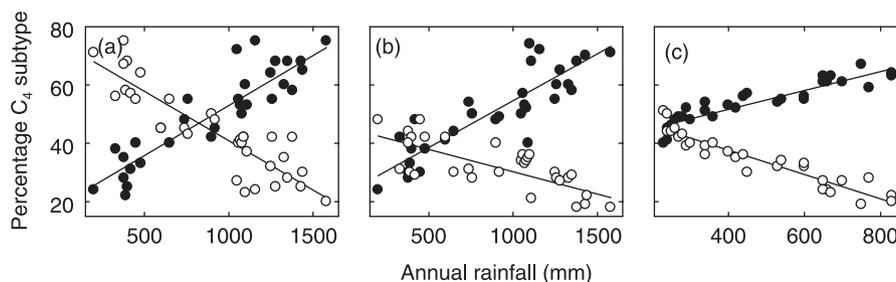


Fig. 5. (a) Percentage of C_4 grasses in US floras belonging to the Panicoideae (●) and the Chloridoideae (○) subfamilies (Taub 2000). (b) Percentage of C_4 grasses in floras with the NADP-ME (●) and NAD-ME (○) subtypes in the USA (Taub 2000) and (c) Australia (Hattersley 1992). Because each flora covers a differing geographical area, values are expressed as a percentage to normalize for species–area biases. (a) and (b) express the same data set on a different basis, excluding (a) members of the Aristidoideae, and (b) species with the PCK subtype of C_4 photosynthesis.

The mechanisms underpinning these relationships are unclear at present, but may be linked to the close association between biochemical subtypes of *C*₄ photosynthesis and specific phylogenetic groups; the majority of Panicoideae species utilize the NADP-malic enzyme (NADP-ME) pathway whereas most members of the Chloridoideae use the NAD-malic enzyme (NAD-ME) pathway (Taub 2000). As a consequence, contrasting correlations with rainfall are also recognized for different *C*₄ subtypes (Fig. 5b,c; e.g. Hattersley 1992; Taub 2000; Cabido *et al.* 2007). Experimental investigations suggest that these alternative forms of the pathway may be differentially adaptive in relation to water availability (Ghannoum *et al.* 2002), or a correlated environmental variable such as soil nutrient status (Ghannoum *et al.* 2005). However, none of these experiments have explicitly controlled for phylogeny, and the issue remains unresolved. Further questions remain about how these species richness patterns translate into plant abundance along rainfall gradients.

Despite these uncertainties, the geographical distributions of modern *C*₄ grasses indicate that changing rainfall patterns will drive significant shifts in the phylogenetic composition of *C*₄ grass communities. This observation has important implications for palaeoclimate hypotheses about the Miocene–Pliocene expansion of *C*₄ grasslands, because it allows predictions about the clades of *C*₄ grasses that are involved. First, the range of Middle Miocene ecosystems along inferred rainfall gradients (Retallack 2001) should contain varying proportions of *C*₄ grass subfamilies, following the qualitative patterns in Fig. 5(a). Secondly, a shift in the woodland–grassland ecotone to higher rainfall areas should increase the proportion of *C*₄ species belonging to the Panicoideae relative to the Chloridoideae. Finally, a decrease in the total amount of rainfall should drive the reverse pattern, favouring species of the Chloridoideae relative to the Panicoideae.

These opposing predictions are especially critical for regions such as East Africa and South Asia, where alternative hypotheses are postulated. Crucially, they can be tested by using emerging techniques for quantifying the relative abundance of different grass subfamilies within fossil phytolith assemblages (e.g. Prasad *et al.* 2005), providing a new means of evaluating alternative hypotheses using geological evidence. Conversely, the use of these techniques to reconstruct the phylogenetic make-up of Miocene grassland communities in the Great Plains could offer vital clues about the environmental drivers of *C*₄ grassland expansion in this region. Finally, quantifying shifts in the abundance of different grass subfamilies could aid in the interpretation of carbon isotope signals. For example, a decrease in the proportion of Chloridoideae (NAD-ME) relative to Panicoideae (NADP-ME) species would be expected to ‘amplify’ the *C*₄ signal, because discrimination against ¹³C is stronger in NAD-ME than NADP-ME grasses (Hattersley 1982), i.e. the NAD-ME type is slightly more ‘*C*₃-like’. The power of these approaches would be increased greatly by a better understanding of how *C*₄ grass distributions vary in relation to rainfall seasonality and fire frequency.

Re-evaluating the role of seasonal rainfall

Previous studies suggest that species richness of the tribes Andropogoneae and Paniceae (Fig. 1; Panicoideae) may change significantly in response to the intensity and seasonality of rainfall. For southern Africa, Gibbs-Russell (1988) used a qualitative comparison of floristic and climatic data to show that Andropogoneae species account for the largest fraction of the Panicoideae in monsoonal summer rainfall areas where mean annual precipitation (MAP) > 500 mm. The opposite pattern occurs in the Paniceae, which account for the largest proportion of Panicoideae species in more arid summer-rainfall regions (MAP < 500 mm).

Hartley (1950, 1958a,b) carried out a similar analysis at the global scale, but expressed species numbers in each tribe as a fraction of the total grass flora, rather than restricting the analysis to the subfamily Panicoideae. Based on qualitative descriptions of maps, Hartley hypothesized that centres of the highest species diversity in the Andropogoneae are typically located in subtropical monsoon climates with a short rainy season (Hartley 1958a), whereas the highest diversity in the Paniceae occurs in less seasonal wet equatorial climates (Hartley 1958b). To evaluate this hypothesis further, I have carried out a quantitative analysis of the same species richness data sets (Hartley 1950, 1958a) by using climate data recorded at nearby meteorological stations (Müller 1982). A series of generalized linear models (GLIMs) were fitted to the data, using a quasibinomial distribution in place of the binomial distribution when data were underdispersed [glm(), R version 2.4.1, The R Foundation for Statistical Computing]. I used a forward stepwise approach, testing first the simple additive hypotheses posed in the literature, and then adding more variables and interactions.

Numerous previous studies have demonstrated that temperature is the primary global control on species richness for *C*₄ grasses (reviewed by Sage *et al.* 1999), and the Andropogoneae and Paniceae tribes followed this general pattern (Table 2a,b). GLIMs for Eurasia–Africa and the Americas showed highly significant additive effects of mean annual temperature (MAT) and tribe on relative species richness (Fig. 6a,b, Table 2a,b). Relative diversity was lower in the Andropogoneae than the Paniceae (Table 2a,b), and this difference was more pronounced in the Americas than Eurasia and Africa (Fig. 6a,b).

I then added rainy season length (RSL) to the model, restricting the analysis to tropical and subtropical localities (latitude < 35°N or S). Because the latitude criterion excluded most of the data from the Americas, this analysis focused on Eurasia and Africa. RSL was estimated for each locality using a biologically based definition of the growing season for *C*₄ grasses; as the number of consecutive months in which the mean minimum temperature > 10 °C and total precipitation > 30 mm (Collatz *et al.* 1998). The temperature threshold for growth was based upon two lines of complementary evidence: (i) experimental observations of chilling-mediated photo-inhibition and impairment of leaf extension in *C*₄ grasses (Long 1983); and (ii) the minimum summer temperature required

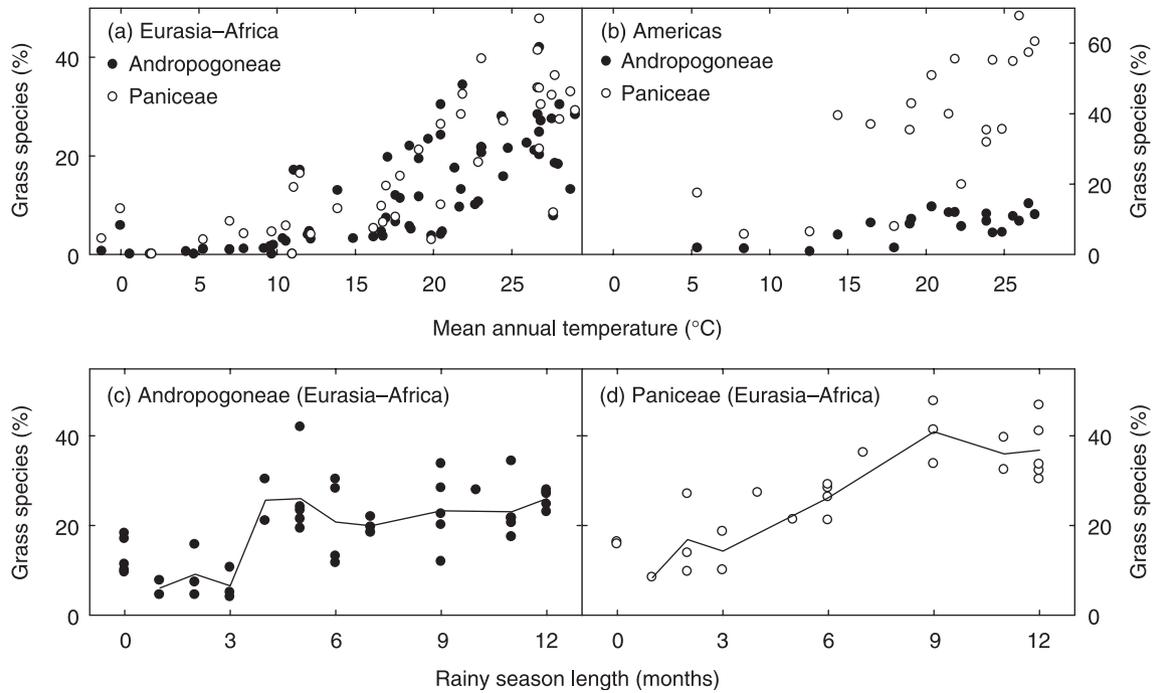


Fig. 6. Global variation in relative species richness within the grass subfamily Panicoideae, showing species abundance within the tribes Andropogoneae and Paniceae as a proportion of all grass species in a flora (%), relative to climatic variables. The upper panels show variation due to mean annual temperature for (a) Eurasia and Africa and (b) North and South America, and the lower panels show variation due to rainy season length (RSL) in Eurasia and Africa for (c) the Andropogoneae and (d) the Paniceae, with lines indicating mean monthly values.

Table 2. Generalized linear models (GLIMs) explaining the global variation in relative species richness within the grass subfamily Panicoideae. The models consider the interacting effects of mean annual temperature (MAT), rainy season length (RSL) and tribe (Andropogoneae or Paniceae).

Factor	d.f.	<i>F</i>	<i>P</i>
(a) Americas			
MAT	1,38	35.9	< 0.0001
Tribe	1,37	120.6	< 0.0001
MAT × Tribe	–	–	NS
(b) Eurasia–Africa			
MAT	1,112	170.2	< 0.0001
Tribe	1,111	8.1	0.0053
MAT × Tribe	–	–	NS
(c) Eurasia–Africa			
MAT	1,59	40.1	< 0.0001
RSL	1,58	25.4	< 0.0001
Tribe	1,57	6.1	0.0162
MAT × Tribe	–	–	NS
RSL × Tribe	–	–	NS
(d) Eurasia–Africa			
MAT	1,59	61.4	< 0.0001
RSL (binned)	1,57	31.8	< 0.0001
Tribe	1,56	12.7	0.0008
RSL × Tribe	2,54	3.1	0.0549
MAT × Tribe	–	–	NS

for C_4 species to persist in a grass flora (Sage *et al.* 1999), a threshold that was observed in this data set. These criteria defined a reasonable (non-zero) rainy season for all localities except those in desert regions (e.g. Syria and Yemen), where

plants are probably associated with bodies of water or infrequent rains, or at high elevation (e.g. Lesotho), where C_4 species may show an unusual resistance to low temperature extremes (Márquez *et al.* 2007). These localities with a zero RSL were excluded from further analysis.

The minimum adequate GLIM showed highly significant additive effects of MAT, RSL and tribe, but not the hypothesized interaction between MAT and RSL (Table 2c). Species richness increased with RSL in both tribes, but showed a differing nonlinear response: values for the Andropogoneae were low when $0 < \text{RSL} \leq 3$ months, and high when $4 \leq \text{RSL} \leq 12$ months (Fig. 6c); in contrast, values for the Paniceae increased in an approximately linear response to reach a maximum at $\text{RSL} \geq 9$ months (Fig. 6d). I therefore constructed a new GLIM attempting to account for these apparent nonlinearities by grouping RSL data into three temporal categories: 1–3, 4–7 and 9–12 months (there were no localities with $\text{RSL} = 8$ months). This model showed an additive effect of MAT, and an interaction between RSL and tribe (Table 2d). The interaction was caused by equal species richness in climates with a rainy season of 7 months or less, and greater species richness in the Panicoideae than Andropogoneae in aseasonal climates with rainy seasons of 9 months or more (Fig. 7).

This quantitative analysis supports the hypothesis that species richness in the Paniceae is highest in moist, aseasonal climates of the subtropics and tropics, but fails to show a similar association between maximal Andropogoneae diversity and a short rainy season. Instead, species richness in the Andropogoneae remains constant across localities with rainy

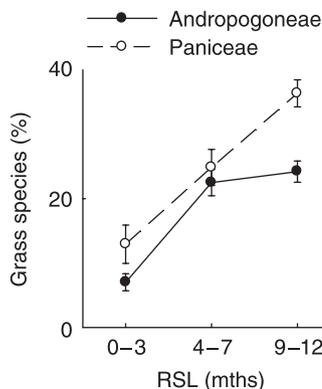


Fig. 7. Interaction between species richness in the Andropogoneae and Paniceae tribes and rainy season length (RSL) in Eurasia and Africa. Species abundance within each tribe is expressed as a proportion of all grass species in a local flora (%), and shown as the mean \pm SE for each RSL category.

seasons of 4–12 months (Figs 6c and 7). The analysis therefore highlights a phylogenetic contrast between the tribes that could prove useful for hypothesis testing in the geological record: a climatic change from moist, aseasonal conditions to a strongly seasonal climate is expected to drive a shift in the grass flora from a predominance of Paniceae species towards a more equal representation of the Paniceae and Andropogoneae. Further work is required before this expectation can be tested in the fossil record, because techniques for analysing phytolith assemblages cannot yet distinguish tribes of the Panicoideae. However, archaeologists already use phytolith-based diagnostics for identifying individual crop species of the Andropogoneae (Piperno 2006), indicating good promise for increasing the taxonomic resolution of this technique.

The value of these observed patterns of species richness would be increased greatly by an understanding of the underlying mechanisms. One possibility is suggested by Bond *et al.* (2003), who hypothesize that the Andropogoneae are key to the fire-mediated displacement of forests by grasslands in mesic climates of southern Africa. These authors note the dominance of this group in fire-maintained and nutrient-poor mesic savannas, the obligate dependence of Andropogoneae species such as *Themeda triandra* on frequent defoliation, and their decline in the absence of fires. Frequent fires are promoted by the rapid growth of Andropogoneae species during summer, the accumulation of tannin-like compounds in their foliage, and the low nutritional quality of leaves during winter (reviewed by Bond *et al.* 2003). These latter traits retard decomposition and result in a low palatability to grazers during the dry season, leading to the build up of a high fuel load for fires.

The evolution of such fire-promoting traits in one or more clades of *C*₄ grasses during the Miocene therefore provides a possible explanation for how the grassland–woodland ecotone shifted to more mesic areas. But what might have selected for these characters? One idea is suggested by the Miocene radiation of grazers, and the specialization of some

animals on a *C*₄ diet prior to grassland expansion. Recent studies indicate that modern grasses have evolved a suite of characteristics to resist grazing, including the accumulation of phenolic compounds (e.g. tannins), a high silica content, and tough fibrous leaves with a low palatability and nutritional quality (Burt-Smith *et al.* 2003; Massey *et al.* 2007). Ecological theory proposes that selection for these traits is strongest in nutrient-poor habitats (Grime 2001), and empirical evidence shows that grazer-driven selection for unpalatable species occurs in dry ecosystems with a long history of herbivory (Díaz *et al.* 2007). Speculative links may therefore be drawn between high grazing pressure in nutrient-poor savannas during the Miocene, and selection for grazing-resistant (but fire-promoting) traits in *C*₄ grasses. As these traits may also occur in *C*₃ grasses (Grigulis *et al.* 2005), this hypothesis provides an ecological explanation for Miocene *C*₄ grassland expansion that is not directly linked to photosynthetic pathway.

Conclusions

The origin of *C*₄ photosynthesis and expansion of *C*₄ grasslands were major events in Earth history with significant consequences for tropical and subtropical ecology. Newly uncovered geological evidence has bolstered support for decreasing CO₂ as a selection agent for the pathway, leading to the expectation that *C*₄ grasses first evolved during the Oligocene. However, these same data cast serious doubt on the role of CO₂ in *C*₄ grassland expansion. Instead, current ecological understanding of vegetation–fire dynamics in savannas has generated hypotheses integrating the roles of climate change and fire. I propose that the contrasting modern diversity patterns shown by independent *C*₄ grass clades along climatic gradients generate a further expectation: that palaeoclimate change forced significant shifts in the phylogenetic composition of Miocene grass communities. New techniques for the geochemical analysis of pollen and the extraction of phylogenetic information from phytolith assemblages provide the necessary tools for testing these ideas, and will help to bring the evolutionary history of *C*₄ grasses into sharper focus.

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